## REMARKS

### I. Status of the Claims

Claims 15-40 are pending in this application. Claims 1-14 were previously cancelled. Claims 18-26 have been amended to more particularly point out and distinctly describe the claimed subject matter. Claims 15-17 and 28-40 have been cancelled without prejudice or disclaimer. Applicants expressly reserve the right to pursue any cancelled subject matter in subsequent applications that claim benefit from this application.

Claim 18 has been amended to recite "CMP-N-acetylneuraminic acid hydroxylase (CMAH)." Support for this amendment may be found on page 2 of publication WO/2007/0154982. Claim 18 has also been amended to recite "wherein said portion comprises the sequence including and disposed between...". Claim 20 has been similarly amended. Support for these amendments may be found throughout the specification and in particular in paragraphs 18-19 of the specification at US 2007/0154982.

Claims 19-25 have been amended to recite "the CHO cell" instead of "a CHO cell."

Claim 21 has been amended to recite "wherein said portion encodes the sequence: SEQ ID NO:2."

Claims 23, 24, and 26 have been amended to depend from claim 18 in view of the cancellation of claim 15.

No new matter has been introduced. Upon entry of the present amendment, claims 18-27 will be pending and under examination in the instant application. Reconsideration is respectfully requested.

## II. Rejections Under 35 U.S.C. §112, (Indefiniteness)

Claims 15-40 have been rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter of the present invention.

First, the Examiner states that claim 15 does not clearly identify "CMAH" as meaning "CMP-N-acetylneuraminic acid hydroxylase." The Examiner also states that the term "CNAH" is used to identify the same protein (See Fontana et al. 1999, Animal Cell Technology, p. 245-249).

It is noted at the outset that Applicants have cancelled claims 15-17 and 28-40 without prejudice or disclaimer, rendering the rejections of these claims moot.

Claim 18 has been amended to recite: "CMP-N-acetylneuraminic acid hydroxylase (CMAH)." Support for this amendment may be found throughout the specification and in particular in paragraph 11 of the specification at US 2007/0154982.

The Examiner also states that claims 16-25 and 29-38 are unclear in reciting "a CHO cell." In response, claims 16-25 have been amended to recite "the CHO cell."

In claim 18, the Examiner finds the language "said portion is within encoding for the sequence disposed between bases" confusing and unclear. The Examiner states that is not clear if the deleted portion is within the sequence between the bases 787 and 1598 or if the deleted portion includes bases 787 and 1598. Claim 18 has been amended to recite "wherein said portion comprises the sequence including and disposed between..." to clarify that the portion includes bases 787 and 1598. Claim 20 has been similarly amended. Support for these amendments may be found throughout the specification and in particular in paragraphs 18-19 of the specification at US 2007/0154982.

Claim 21 has been rejected as failing to have proper antecedent basis with respect to SEQ ID NO:2. In response, claim 21 has been amended to recite "wherein said portion

encodes the sequence of SEQ ID NO:2" to provide the proper reference for the amino acid sequence.

In view of the present amendments, Applicants respectfully request that the rejections under 35 U.S.C. § 112, second paragraph be withdrawn.

## III. Rejections Under 35 U.S.C. §102/103

Claims 15-40 have been rejected under 35 U.S.C. 102(b) as being anticipated by or, in the alternative under 35 U.S.C. 103(a) as being obvious over Fontana *et al.* 1999, *Animal Cell Technology*, p. 245-249.

The Examiner cites Fontana for making CMAH knock-outs of CHO cells using homologous recombination. The Examiner also states that Fontana abbreviates the gene CMAH as CNAH (referencing page 245). The Examiner further states that Fontana describes CHO cells which comprise a knock-out of the coding sequence for cytochrome b5 binding site and of the binding site for CNAH.

Applicants note that Fontana describes only certain specific plasmid constructs for making CMAH knockouts where the deleted portion of the hamster CMAH gene is predicted to span the functional domains for the Rieske center and for the first mononuclear iron binding site (i.e., the deleted regions would span portions of exon 2 and exon 5, See Fig. 1-2). Fontana does not describe or suggest knocking out exons other than exons 2 and 5. Fontana only identified a portion of the hamster CMAH coding regions as spanning putative exons 2-9 as aligned with the known mouse, human, and pig CMAH sequences as shown in Fontana Figs. 1-2. Fontana only describes a partial cDNA coding sequence showing the deduced amino acid sequence containing residues 1-366 (See fig. 2). Thus, Fontana provides no teachings with respect to a longer hamster CMAH cDNA sequence including exons 10-15, and no teachings with respect to a hamster CMAH protein of 564 residues.

Fontana also fails to teach knocking out exons 8 and 9. Instead, Fontana comments only on a naturally occurring deletion mutant in a murine CNAH (citing Koyama *et al.* 1996,

Glycoconj. J. (13):353-358) as corresponding to exon 8 in the hamster sequence. Thus, Fontana does not teach or suggest CHO cells with knock-outs of portions of exons 8 and 9.

In contrast to Fontana, the amended claims require knocking out specific portions of the genes between exons 8 and 15, *i.e.*, the portion comprising the sequence including and disposed between bases 787 and 1598 of cDNA encoding for CMAH (and also the equivalent portion comprising the gene encoding for the sequence of CMAH including and disposed between amino-acid 262 and amino-acid 532).

Since Fontana does not describe any CMAH knock-outs other than plasmid constructs for making putative CMAH knockouts where the deleted portion of the hamster CMAH gene would span portions of exon 2 and exon 5, Fontana cannot anticipate the claimed CHO cells containing a deletion comprising the sequence including and disposed between bases 787 and 1598 of cDNA encoding for CMAH or CHO cells containing the equivalent portion comprising the gene encoding for the sequence of CMAH including and disposed between amino-acid 262 and amino-acid 532.

Exhibit 1 attached herewith is an alignment illustrating the differences between the putatively deleted sequences of Fontana and the region deleted in the claimed cell lines. The SEQ ID:2 amino acid sequences correspond to the cDNA segment that spans and includes bases 787-1598 of the CMAH coding region. The alignment clearly shows that the deletion described by Fontana is different and does not overlap with the regions presently claimed.

Furthermore, it is noted that gene targeting by homologous recombination in mammalian somatic cells is difficult and unpredictable. The present invention provides plasmids containing specific constructs that provide efficient removal of unwanted CMAH enzymatic activity in a mammalian cell strain (CHO cells) by gene targeting. The resulting CHO cells are useful for expression of recombinant glycoproteins. The specification also provides techniques for deletion of both alleles by repeated transfection of the same homologous recombination plasmid and subsequent removal of selection marker cassettes needed for clone selection.

In contrast, Fontana merely describes a theoretical approach for gene targeting based on one plasmid for gene homologous recombination which contains a portion of the hamster CMAH coding sequence (a different portion from the portions claimed in the present application, as described above), with no precisely defined deletion boundaries and without any experimental description relating to obtaining the recombinant CHO cells, and without any confirmation of results.

Finally, the rejections of claims 28-40 are moot in view of the cancellation of these claims.

For at least the reasons set forth above, claims 18-27 are not anticipated or obvious over the prior art of record. Reconsideration of claims 18-27 and withdrawal of the rejections under 35 U.S.C.§§ 102(b) and 103(a) is requested.

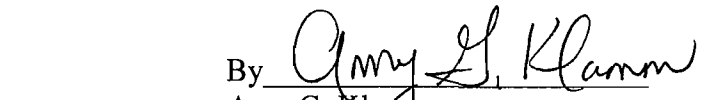
## CONCLUSION

In view of the above amendments and remarks, it is respectfully requested that the application be reconsidered and that all pending claims be allowed and the case passed to issue. Applicants reserve the right to pursue the canceled and/or non-elected subject matter in one or more continuation or divisional applications.

If there are any other issues remaining, which the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Dated: January 29, 2008

Respectfully submitted,

By 

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